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SEMI-ANNUAL PROGRESS REPORT

Report prepared by: R. L. Thompson July 18, 1952

NR: 134-718/2-6-51 (L) For period: Jan. 1 to June 30, 1952

CONTRACT: N6onr-18009

ANNUAL RATE: 7,500

CONTRACTOR: Indiana University

PRINCIPAL INVESTIGATOR: R. L. Thompson

Assistants: Drs. E. Campaigne, H. G. Day, S. A. Minton, Jr.

TITLE OF PROJECT: Nutritional Requirements of Mammalian Viruses.

Objectives: 1) To synthesize substituted amino acids, thyroxine-like substances, thiosemicarbazones and other materials which may influence viral growth. 2) To determine the effect of these and other materials on multiplication of mammalian viruses.

Summary of results:

a) Since start of project: 1) Synthesis of 33 compounds, including analogues of amino acids, thyroxine-like materials, thiosemicarbazones and rhodanines. 2) In vitro experiments with viruses: i) Fluorophenylalanine and thienylalanine inhibit multiplication of vaccinia virus in chick embryonic tissues and the inhibition is counteracted by phenylalanine. ii) 2,6-Diaminopurine inhibits multiplication of vaccinia virus in chick embryonic tissues and the inhibition is overcome by adenine and adenine-containing compounds. iii) 5-(2'4'-Dichlorophenoxy)-4-hydroxy-2-mercaptopyrimidine and related compounds are weak inhibitors of vaccinia virus in chick embryonic tissues. iv) Thiosemicarbazones vary in their capacity to inhibit growth of vaccinia virus in chick or mouse embryonic tissues. 3) In vivo experiments with viruses: i) Mice inoculated intracerebrally with vaccinia (IHD strain) or variola (Williamsport strain) viruses are not protected by treatment with fluorophenylalanine, thienylalanine or 2,6-diaminopurine. ii) The resistance of mice to these viruses is increased by treatment with 5-(2'4'-dichlorophenoxy)-4-hydroxy-2-mercaptopyrimidine or certain thiosemicarbazones. iii) Most thiosemicarbazones which protect mice against variola-vaccinia viruses inhibit growth of vaccinia virus in vitro. The pyruvic acid derivative is highly active in vitro but inactive in animals; cinchoninaldehyde thiosemicarbazone is weakly active in the test tube but quite effective in mice. iv) Death of mice inoculated intracerebrally with Lansing poliomyelitis virus often is delayed by treatment with certain thiosemicarbazones, thiouracils or thyroxine analogues.

b) During current period: 1) Chemical syntheses: Preparation of 24 compounds, including thiosemicarbazones and thiohydantoins. The compounds are listed in the appendix. 2) Biological: i) Effect of dosage, time and route of administration of thiosemicarbazones on protection of mice against variola-vaccinia viruses: Data are presented in tabular form in the appendix. Isatin thiosemicarbazone, administered intraperitoneally, was found to protect mice inoculated intracerebrally with a recently isolated variola virus (Williamsport strain). A single dose of 5-10 mg. is most effective when given 24-48 hours after initiation of infection.

Three doses of 2.5 mg., each, administered within 48 hours after inoculation of virus protects the majority of mice and is equivalent or superior to the feeding of 0.04% of the compound in the diet. Three doses of 1 mg., each, reduces the fatality rate. 2-Methoxybenzaldehyde, 5-nitro-2-thenaldehyde, cinchoninaldehyde and isonicotinic acid thiosemicarbazones also are effective when administered by the peritoneal route. Other compounds have not been teste are too toxic by this route, or are inactive when administered intraperitoneall ii) Effect of thiosemicarbazones on multiplication of variola virus in the brai of the mouse. Mice were inoculated intracerebrally with variola virus (Williamsport strain) and treated by intraperitoneal injection of isatin thiosemicarbazone for 48 hours immediately after inoculation of virus. Treated and untreated mice were sacrificed each day and the viral content of the brains determined by intradermal titration in rabbits. Data from 4 experiments are presented graphically in the appendix. No significant differences in the concentration of virus in the brains of treated and untreated mice was detected Similar results were obtained from experiments in which benzaldehyde or 5-bromo 2-thenaldehyde thiosemicarbazones were fed in the diet. 5-(2'4'-Dichlorophenox 4-hydroxy-2-mercaptopyrimidine may reduce viral multiplication. iii) Semliki Forest virus: This neurotropic virus (Smithburn & Haddow, 1944) was introduced into the study because it produces fatal infection of mice following intraperitoneal injection. Mice inoculated in this manner and fed test materials in the diet were found to react as follows: 1) Ethionine, an analogue of the amino acid methionine, when fed in a strength of 0.15%, resulted in the survival of 41 of 70 mice (60%) as compared to 17 of 99 (17%) untreated animals. 2) 5-(2'4'-Dichlorophenoxy)-4-hydroxy-2-mercaptopyrimidine (0.75%) treatment resulted in th survival of 14 of 32 mice (44%) as compared to 5 of 47 (11%) untreated animals. 3) Thiosemicarbazones (6), semicarbazones (2), purines (10), other pyrimidines (8), analogues of other amino acids and of growth factors either failed to protect mice or possibly decreased resistance. iv) Poliomyelitis virus: No additional experiments.

Plans for Future:

Immediate: 1) To study the effect of treatment with thiosemicarbazones or 5-phenoxy thiouracils on the response of tissues of mice and chick embryos to variola-vaccinia viruses. 2) To determine the effect of prolonged propagation of variola-vaccinia viruses in mice treated with thiosemicarbazones or 5-phenox thiouracils. 3) Investigation of the effect of methionine analogues, 5-phenoxy thiouracils and other substances on Semliki Forest virus infection in mice. 4) Determination of the effect of these materials on infections of mice due to other neurotropic viruses.

REPORTS AND PUBLICATIONS:

R. L. Thompson and S. A. Minton, Jr. "Effect of heterocyclic and other thiosemicarbazones on vaccinia infection in the mouse." Annual meeting, Amer. Assn. Immunologists, New York City, April 14-18, 1952. (Fed. Proc. 11, 485, 1952).

R. L. Thompson, S. A. Minton, Jr., J. E. Officer and G. H. Hitchings, "Effect of heterocyclic and other thiosemicarbazones on vaccinia infection in the mouse."

J. Immunol. Being submitted for publication.

S. A. Minton, Jr., J. E. Officer and R. L. Thompson, "Effect of thiosemicarbazone and dichlorophenoxythiouracil on multiplication of a recently isolated strain of variola-vaccinia virus in the brain of the mouse." J. Immunol. Being submitted for publication.

E. Campaigne and W. L. Archer, "Thiosemicarbazones of 5-substituted isatins." J.A.C.S. Being submitted for publication.

E. Campaigne, P. Monroe, B. Armwine and W. L. Archer, "Thiosemicarbazones of thiophene derivatives." J.A.C.S. Being submitted for publication.

SEMI-ANNUAL PROGRESS REPORT: Supplementary report.

NR: 134-718/2-6-51 (L)

Results obtained during current report period:

1. Chemical syntheses: Since the last report, the following compounds have been prepared and submitted for testing:

ONR 36 5-methyl-2-thenaldehyde thiosemicarbazone
ONR 37 5-nitroisatin thiosemicarbazone
ONR 38 5-bromoisatin thiosemicarbazone
ONR 39 2-thenaldehyde semicarbazone
ONR 40 3-methyl-2-thenaldehyde thiosemicarbazone
ONR 41 9-anthraldehyde thiosemicarbazone
ONR 42 5-(beta-ethylmercaptoethyl)-2-thiohydantoin
ONR 43 5-acetamido-2- enaldehyde thiosemicarbazone
ONR 44 p-dimethylaminobenzaldehyde thiosemicarbazone
ONR 45 isonicotinaldehyde thiosemicarbazone
ONR 46 1-acetyl-5-(p-chlorobenzoyl)-2-thiohydantoin
ONR 47 1-acetyl-5-(p-bromobenzyl)-2-thiohydantoin
ONR 48 1-acetyl-5-(3-thenyl)-2-thiohydantoin
ONR 49 1-acetyl-5-methyl-2-thiohydantoin
ONR 50 1-acetyl-5-(beta-methylmercaptoethyl)-2-thiohydantoin
ONR 51 1-acetyl-5-(beta-ethylmercaptoethyl)-2-thiohydantoin
ONR 52 5,5-diphenylpseudothiohydantoin
ONR 53 5-phenylpseudothiohydantoin.HCl
ONR 54 5-benzylpseudothiohydantoin
ONR 55 5-(p-hydroxybenzyl)-2-thiohydantoin
ONR 56 1-acetyl-5-methyl-5-phenyl-2-thiohydantoin
ONR 57 1-acetyl-5-benzyl-2-thiohydantoin
ONR 58 5-benzyl-2-thiohydantoin
ONR 59 5-t-butyl-2-thenaldehyde thiosemicarbazone.

Table I

Effect of thiosemicarbazones and related compounds on vaccinia infection in the mouse

| Source* | No. | Compound | Conc. in diet(%)** | Proportion of mice surviving in dilution of*** Treated groups $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | Untreated groups $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | Excess surviv in tre ed gro |
|---|---|---|---|---|---|---|---|---|---|---|
| EKC | 1 | thiosemicarbazide | 0.04 | 0/6 | 0/6 | 2/6 | 0/6 | 1/6 | 1/5 | 0 |
| WRL | 2 | pyruvic acid T.S.**** | .1 | 0/6 | 0/6 | 3/6 | 0/6 | 1/6 | 2/6 | 0 |
| " | 3 | cyclohexanone T.S. | 0.06 | 0/6 | 0/6 | 0/6 | 0/5 | 1/6 | 3/6 | -4 |
| " | 4 | benzaldehyde T.S. | 0.04 | 4/6 | 2/6 | 5/5 | 0/5 | 1/6 | 1/5 | 9 |
| | | | 0.08 | 5/5 | 2/5 | 3/5 | 0/5 | 1/6 | 1/5 | 8 |
| " | 5 | benzaldehyde semicarbazone | 0.1 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0 |
| CBC | 6 | p-acetamidobenzaldehyde T.S. | 0.16 | 2/6 | 4/6 | 5/6 | 0/6 | 1/6 | 4/6 | 6 |
| WRL | 7 | p-nitrobenzaldehyde T.S. | 0.04 | 6/14 | 8/11 | 10/12 | 1/11 | 2/12 | 4/12 | 8*** |
| " | 8 | m-nitrobenzaldehyde T.S. | 0.04 | 0/6 | 3/6 | 1/6 | 0/6 | 0/6 | 1/6 | 3 |
| " | 9 | o-methoxybenzaldehyde T.S. | 0.06 | 9/12 | 9/12 | 11/12 | 0/12 | 0/12 | 7/12 | 11*** |
| " | 10 | p-phenylbenzaldehyde T.S. | 0.06 | 0/6 | 0/6 | 0/4 | 0/5 | 1/6 | 3/6 | -4 |
| " | 11 | acetophenone T.S. | 0.04 | 0/5 | 0/6 | 2/6 | 0/5 | 0/6 | 0/6 | 2 |
| " | 12 | propiophenone T.S. | 0.04 | 0/5 | 1/6 | 2/6 | 0/6 | 1/6 | 0/6 | 2 |
| " | 13 | ethylphenylglyoxalate T.S. | 0.15 | 1/7 | 3/6 | 1/6 | 0/8 | 0/6 | 2/6 | 3 |
| " | 14 | desoxybenzoin T.S. | 0.04 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0 |
| " | 15 | phenylacetaldehyde T.S. | 0.04 | 1/6 | 3/6 | 4/6 | 1/6 | 2/6 | 3/6 | 2 |
| " | 16 | cinnamaldehyde T.S. | 0.04 | 0/6 | 0/4 | 0/6 | 0/6 | 1/6 | 1/6 | -2 |
| HR | 17 | picolinaldehyde T.S. | .03 | 0/6 | 1/6 | 0/4 | 0/5 | 1/6 | 3/6 | -3 |
| CBC | 18 | nicotinaldehyde T.S. | .06 | 1/8 | 1/4 | 1/6 | 0/5 | 0/6 | 0/3 | 3 |
| | | | 0.12 | 3/6 | 4/6 | 3/6 | 0/6 | 2/6 | 1/6 | 7 |
| HR | 19 | | 0.08 | 3/6 | 5/6 | 5/6 | 2/6 | 1/6 | 5/6 | 5 |
| " | 20 | isonicotinaldehyde T.S. | 0.08 | 6/6 | 6/6 | 5/6 | 2/6 | 1/6 | 5/6 | 9 |
| | | | 0.08 | - | 5/6 | 5/6 | - | 1/6 | 4/6 | 5 |
| WRL | 21 | cinchoninaldehyde T.S. | 0.02 | 2/6 | 2/6 | 1/6 | 0/6 | 1/6 | 1/6 | 3 |
| | | | 0.04 | 15/18 | 11/17 | 14/18 | 1/18 | 3/18 | 2/18 | 11**** |
| | | | 0.08 | 5/6 | 5/6 | 2/6 | 0/6 | 1/6 | 1/6 | 10 |
| | | | 0.12 | 2/6 | 6/6 | 2/6 | 0/6 | 1/6 | 1/6 | 8 |
| " | 22 | cinchoninaldehyde semicarbazone | 0.04 | 0/5 | 1/6 | 1/5 | 0/6 | 0/6 | 1/6 | 1 |
| | | | 0.15 | 0/8 | 0/6 | 0/6 | 0/5 | 0/6 | 0/3 | 0 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| " | 23 | isatin T.S. | 0.08 | 4/6 | 4/6 | 5/6 | 0/6 | 1/6 | 2/6 | 10 |
| | | | 0.12 | 6/6 | 4/6 | 5/5 | 0/6 | 2/6 | 1/6 | 12 |
| | | | 0.16 | 6/6 | 4/6 | 6/6 | 0/6 | 1/6 | 4/6 | 11 |
| " | 24 | isatin semicarbazone | 0.1 | - | 1/11 | - | - | 1/6 | - | 0 |
| " | 25 | 5-methyl isatin T.S. | 0.1 | 0/6 | 0/6 | 0/6 | 1/5 | 2/6 | 1/6 | -4 |
| IU | 26 | 5-nitro isatin T.S. | 0.1 | 1/6 | 3/6 | 3/6 | 0/5 | 1/6 | 3/6 | 3 |
| " | 27 | 5-bromo isatin T.S. | 0.1 | 1/6 | 4/5 | 5/6 | 0/5 | 1/6 | 3/6 | 6 |
| WRL | 28 | indole-3-aldehyde T.S. | 0.06 | 2/8 | 1/5 | 1/6 | 1/7 | 1/6 | 3/6 | -1 |
| IU | 29 | 2-furfural T.S. | 0.1 | 1/8 | 1/6 | 0/6 | 0/8 | 0/6 | 2/6 | 0 |
| WRL | 30 | fluorenone T.S. | 0.06 | 1/6 | 2/6 | 5/6 | 0/6 | 0/6 | 1/6 | 7 |
| IU | 31 | 2-thenaldehyde T.S. | 0.02 | 4/6 | 2/5 | 3/5 | 0/6 | 0/6 | 4/6 | 5 |
| | | | 0.04 | 1/5 | 5/6 | 3/6 | 0/6 | 0/6 | 4/6 | 5 |
| | | | 0.06 | 5/5 | 3/5 | 5/6 | 1/6 | 1/6 | 1/6 | 10 |
| | | | 0.08 | 6/11 | 7/12 | 7/12 | 0/12 | 0/12 | 0/12 | 10 ***** |
| | | | 0.1 | 2/6 | 4/6 | 6/6 | 0/6 | 0/6 | 4/6 | 8 |
| " | 32 | 2-thenaldehyde semicarbazone | 0.12 | 0/6 | 0/6 | 1/6 | 0/5 | 1/6 | 1/6 | -1 |
| " | 33 | 5-chloro-2-thenaldehyde T.S. | 0.04 | 0/6 | 4/6 | 5/6 | 0/6 | 2/6 | 3/6 | 4 |
| | | | 0.06 | 5/7 | 4/5 | 2/6 | 0/8 | 1/6 | 0/6 | 10 |
| | | | 0.08 | 5/6 | 6/6 | 6/6 | 0/6 | 1/6 | 4/6 | 12 |
| " | 34 | 5-bromo-2-thenaldehyde T.S. | .02 | 6/6 | 4/6 | 4/6 | 0/6 | 1/6 | 4/6 | 9 |
| | | | 0.06 | 7/13 | 10/12 | 8/11 | 0/11 | 0/12 | 1/9 | 12***** |
| | | | 0.1 | 6/6 | 5/5 | 6/6 | 0/6 | 2/6 | 1/6 | 14 |
| " | 35 | 5-nitro-2-thenaldehyde T.S. | 0.08 | 2/6 | 2/6 | 6/6 | 0/6 | 2/6 | 3/6 | 5 |
| " | 36 | 5-methyl-2-thenaldehyde T.S. | 0.06 | 0/6 | 2/5 | 1/6 | 0/6 | 0/6 | 1/6 | 2 |
| " | 37 | 3-methyl-2-thenaldehyde T.S. | 0.08 | 3/6 | 3/6 | 5/6 | 0/5 | 1/6 | 1/6 | 9 |
| " | 38 | 3-thenaldehyde T.S. | 0.04 | 5/24 | 6/24 | 12/24 | 4/23 | 2/23 | 7/23 | 2***** |
| | | | 0.1 | 4/6 | 0/5 | 1/6 | 1/7 | 1/6 | 3/6 | 0 |
| " | 39 | 2-bromo-3-thenaldehyde T.S. | 0.06 | 4/12 | 7/12 | 6/11 | 1/12 | 3/12 | 2/12 | 5***** |
| " | 40 | 2-chloro-3-thenaldehyde T.S. | 0.06 | 3/13 | 1/12 | 6/12 | 1/12 | 1/12 | 1/9 | 3***** |
| " | 41 | 2,5-dichloro-3-thenaldehyde T.S. | 0.1 | 0/6 | 0/5 | 3/6 | 0/6 | 1/6 | 3/6 | -1 |

* Source of materials: EKC-Eastman Kodak Co.; WRL-Dr. G. H. Hitchings, Wellcome Research Laboratories; IU-Dr. E. Campaigne, Indiana University; HR-Dr. J. A. Aeschlimann, Hoffmann-La Roche, Inc.; CBC-Chemical-Biological Coordination Center National Research Council.

** Mice were placed on casein-sucrose diet containing concentration of test material listed for 1-2 days before inoculation of virus and the treatment was continued for 9-13 days. Untreated mice were fed the same basal diet.

*** Virus inoculated intracerebrally.

**** T.S. denotes thiosemicarbazone.

***** Composite of 2 or more experiments; average number of excess survivors per experiment is listed.

TABLE I

Effect of thiosemicarbazones and related compounds on Williamsport virus infection in the mouse.

| Source* | No. | Compound | Treatment** Dose | Route | Duration (days) | Proportion of mice surviving at dilution*** Treated groups $10^{-4}$ | $10^{-5}$ | $10^{-6}$ | Untreated groups $10^{-4}$ | $10^{-5}$ | $10^{-6}$ | Excess Survivors in treated groups |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WRL | 1 | benzaldehyde T.S.**** | 0.06 | In diet | -3 to 10 | 5/8 | 2/6 | 1/6 | 0/7 | 0/6 | 0/6 | 8 |
| " | 2 | benzaldehyde semicarbazone | 0.1 | " " | -1 to 10 | 0/6 | 1/6 | 0/6 | 0/6 | 0/5 | 1/6 | 0 |
| " | 3 | p-nitro benzaldehyde T.S. | 0.04 | " " | -3 to 10 | 2/6 | 2/6 | 5/6 | 0/7 | 0/6 | 0/6 | 9 |
| " | 4 | o-methoxy benzaldehyde T.S. | 0.06 | " " | -1 to 10 | 0/6 | 2/6 | 5/6 | 0/6 | 0/5 | 1/6 | 6 |
| | | | 5 mg | I.P. | 0,1,2 | 0/6 | 0/6 | 3/6 | 1/6 | 0/6 | 1/6 | 1 |
| NC | 5 | p-isobutoxy benzaldehyde T.S. | 5 mg | " | 0,1,2 | 0/3 | 0/6 | 0/6 | 1/6 | 0/6 | 1/6 | -2 |
| CBC | 6 | p-acetamido benzaldehyde T.S. | 5 mg | " | 0,1,2 | 2/6 | 1/5 | 0/5 | 1/6 | 0/6 | 1/6 | 1 |
| Lo | 7 | 2-thenaldehyde T.S. | 0.06 | In diet | -3 to 10 | 5/7 | 6/6 | 6/6 | 0/7 | 0/6 | 0/6 | 17 |
| | | | 0.06 | " " | -2 to 11 | 3/6 | 4/6 | 5/6 | 0/6 | 1/6 | 0/6 | 11 |
| | | | 0.06 | " " | 0 to 11 | 1/6 | 2/5 | 4/6 | 0/6 | 1/6 | 0/6 | 6 |
| | | | 0.06 | " " | 2 to 11 | 0/5 | 2/5 | 4/6 | 0/6 | 1/6 | 0/6 | 5 |
| | | | 0.02 | " " | -2 to 10 | 2/5 | 4/6 | 5/6 | 2/6 | 0/6 | 2/6 | 7 |
| | | | 0.06 | " " | -2 to 10 | 5/6 | 6/6 | 5/6 | 2/6 | 0/6 | 2/6 | 12 |
| | | | 0.08 | " " | -2 to 10 | 1/4 | 5/6 | 5/6 | 2/6 | 0/6 | 2/6 | 7 |
| | | | 0.12 | " " | -2 to 10 | 2/6 | 6/6 | 6/6 | 2/6 | 0/6 | 2/6 | 10 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| " | 8 | 2-thenaldehyde semicarbazone | 0.12 | " | " | -1 to 10 | 0/6 | 0/6 | 1/6 | 0/6 | 0/5 | 1/6 | 0 |
| " | 9 | 5-chloro-2-thenaldehyde T.S. | 0.06 | " | " | -3 to 10 | 4/7 | 4/6 | 5/6 | 0/7 | 0/6 | 0/6 | 13 |
| " | 10 | 5 bromo-2-thenaldehyde T.S. | 0.1 | " | " | -2 to 10 | 6/6 | 4/6 | 5/5 | 0/6 | 1/6 | 1/6 | 13 |
| | | | 0.1 | " | " | -2 to 2 | 6/6 | 5/6 | 1/6 | 0/6 | 1/6 | 1/6 | 10 |
| | | | 0.1 | " | " | 0 to 4 | 2/6 | 6/6 | 2/6 | 0/6 | 1/6 | 1/6 | 8 |
| | | | 0.1 | " | " | 2 to 6 | 3/6 | 5/6 | 5/6 | 0/6 | 1/6 | 1/6 | 11 |
| " | 11 | 5-nitro-2-thenaldehyde T.S. | 0.1 | " | " | 0 to 10 | 2/6 | 4/6 | 5/6 | 0/6 | 2/6 | 0/6 | 9 |
| | | | 5 mg | I.P. | | 1,2 | 2/6 | 3/5 | 6/6 | 2/6 | 0/6 | 2/6 | 7 |
| " | 12 | 2-acetamido-2-thenaldehyde T.S. | 0.06 | In diet | | -1 to 10 | 1/6 | 0/6 | 2/6 | 1/6 | 0/6 | 1/6 | 1 |
| " | 13 | 5-methyl-2-thenaldehyde T.S. | 0.1 | " | " | -1 to 10 | 2/6 | 3/6 | 2/6 | 0/6 | 0/5 | 1/6 | 6 |
| " | 14 | 3-methyl-2-thenaldehyde T.S. | 0.1 | " | " | -1 to 10 | 4/6 | 5/6 | 6/6 | 1/6 | 0/6 | 1/6 | 13 |
| WRL | 15 | cinchoninaldehyde T.S. | 4 mg | I.P. | | -2 to 8 | 3/6 | 6/6 | 5/6 | 0/6 | 1/6 | 1/6 | 12 |
| " | 16 | cinchoninaldehyde semicarbazone | 0.1 | In diet | | -2 to 10 | 5/6 | 4/6 | 3/6 | 2/6 | 0/6 | 2/6 | 8 |
| " | 17 | isatin T.S. | 0.02 | " | " | -1 to 8 | 2/6 | 1/6 | 3/6 | 0/5 | 0/6 | 1/6 | 5 |
| | | | 0.04 | " | " | -1 to 8 | 2/6 | 5/6 | 4/6 | 0/5 | 0/6 | 1/6 | 10 |
| | | | 0.08 | " | " | -1 to 8 | 3/6 | 4/6 | 3/5 | 0/5 | 0/6 | 1/6 | 9 |
| | | | 0.12 | " | " | -1 to 8 | 3/6 | 5/6 | 4/6 | 0/5 | 0/6 | 1/6 | 11 |
| | | | 10 mg | I.P. | | 1 | 2/6 | 6/6 | 5/6 | 1/6 | 0/6 | 0/6 | 12 |
| | | | 10 mg | " | | 2 | 4/6 | 6/6 | 5/6 | 1/6 | 0/6 | 0/6 | 14 |
| | | | 10 mg | " | | 3 | 3/6 | 1/6 | 3/6 | 1/6 | 0/6 | 0/6 | 6 |
| | | | 5 mg | " | | 1,2 | 4/6 | 6/6 | 5/6 | 0/6 | 0/6 | 1/5 | 14 |
| | | | 5 mg | " | | 2 | 0/4 | 3/6 | 4/6 | 0/6 | 0/6 | 1/5 | 6 |
| | | | 5 mg | " | | 3,4 | 0/6 | 1/6 | 5/6 | 0/6 | 0/6 | 1/5 | 5 |
| | | | 5 mg | " | | 0,1,2 | 6/6 | 6/6 | 5/6 | 0/5 | 0/6 | 1/6 | 16 |
| | | | 2.5 mg | " | | 0,1,2 | 6/6 | 6/6 | 4/5 | 0/5 | 0/6 | 1/6 | 15 |
| | | | 1 mg | " | | 0,1,2 | 6/6 | 3/6 | 3/6 | 0/5 | 0/6 | 1/6 | 11 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IU | 18 | 5-nitro isatin T.S. | 5 mg | " | 0,1,2 | 1/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 1 |
| " | 19 | 5-bromo isatin T.S. | 5 mg | " | 0,1,2 | 0/6 | 0/6 | 1/6 | 0/6 | 0/6 | 0/6 | 1 |
| WRL | 20 | 5-methyl isatin T.S. | 0.1 | In diet | -1 to 10 | 0/6 | 2/6 | 1/6 | 0/6 | 0/5 | 1/6 | 2 |
| " | 21 | fluorenone T.S. | 0.06 | " " | 0 to 7 | | 1/11 | | | 0/6 | | |
| " | 22 | dichlorophenoxy thiouracil | 0.75<br>20 mg | " "<br>I.P. | -1 to 10<br>1,2 | 2/6<br>2/6 | 2/6<br>4/5 | 3/6<br>1/3 | 0/6<br>2/6 | 0/5<br>0/6 | 1/6<br>2/6 | 6<br>3 |
| " | 23 | 2-mercapto-4-hydroxy-5-(p-chlorophenyl)-2-mercapto-pyrimidine | 20 mg | " | 1,2 | 0/5 | 1/5 | 2/6 | 2/6 | 0/6 | 2/6 | -1 |
| " | 24 | p-methoxy-ω-chloro-acetanilide | 10 mg | " | 1 | 0/6 | 1/4 | 0/5 | 0/6 | 2/6 | 0/6 | -1 |
| " | 25 | 2,6-diamino-8-p-chlorophenyl purine | 10 mg | " | 1 | 0/6 | 0/5 | 0/6 | 0/6 | 2/6 | 0/6 | -2 |
| R | 26 | isonicotinic acid | 1.5 | In diet | 0 to 9 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0 |
| WRL | 27 | isonicotinic acid hydrazide | 0.1 | " " | 0 to 9 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0 |
| " | 28 | isatin-beta-hydrazone | 0.1<br>5 mg | " "<br>I.P. | 0 to 9<br>0,1,2 | 0/6<br>0/6 | 0/6<br>0/6 | 1/6<br>0/6 | 0/6<br>0/6 | 0/6<br>0/6 | 0/6<br>0/6 | 1<br>0 |

* Source of materials: WRL - Dr. G. H. Hitchings, Wellcome Research Laboratories; IU - Dr. E. Campaigne, Indiana University; MC - Dr. A. F. Langlykke, E. R. Squibb & Sons; CBC - Chemical-Biological Coordination Center, National Research Council; R - Reilly Tar & Chemical Corp.

** Treatment: Doses expressed in terms of mg were given by intraperitoneal injection; in other cases the concentration in per cent of the compound fed in the diet is listed. I.P. denotes injection by peritoneal route. Negative values listed under duration of treatment indicate the number of days of treatment before inoculation of virus. Animals treated parenterally received a single injection each day; the days on which injections were made are listed.

*** Virus inoculated intracerebrally.

**** T.S. denotes thiosemicarbazone.